Supplemental Data

REGULATION OF FIBROBLAST GROWTH FACTOR-23 SIGNALING BY KLOTHO

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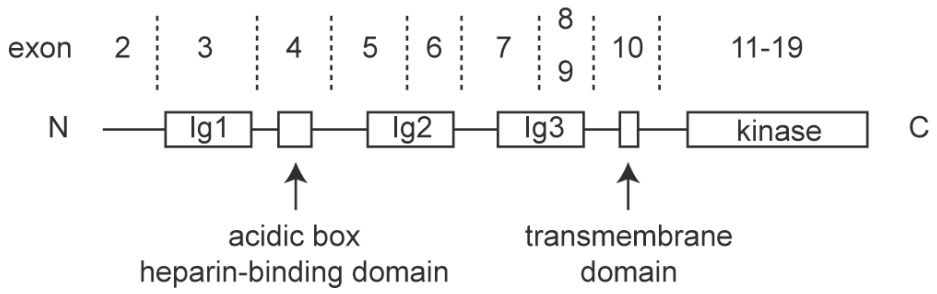


FGFR1

Exon#	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
bL	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+
bS	+	+	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+
cL	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+
cS	+	+	–	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+

FGFR2

Exon#	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
bL	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+
bM	+	+	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+
bS	+	+	–	–	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+
cL	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+
cM	+	+	–	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+
cS	+	+	–	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+

FGFR3

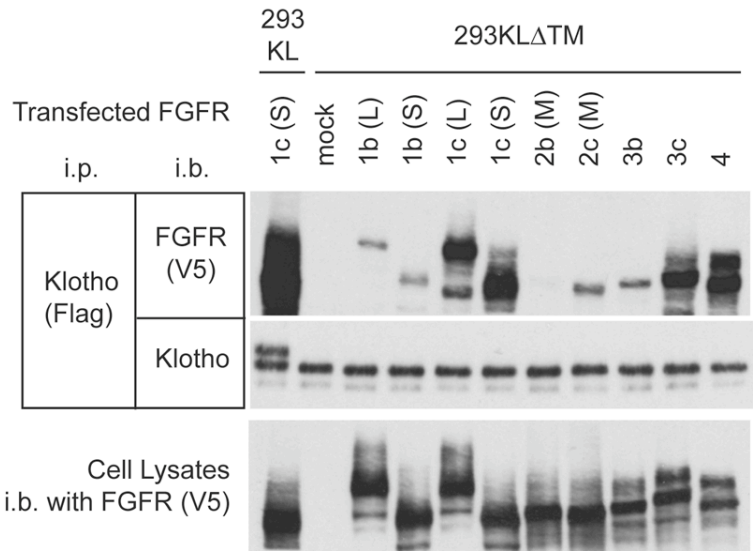
Exon#	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
b	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+
c	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+

FGFR4

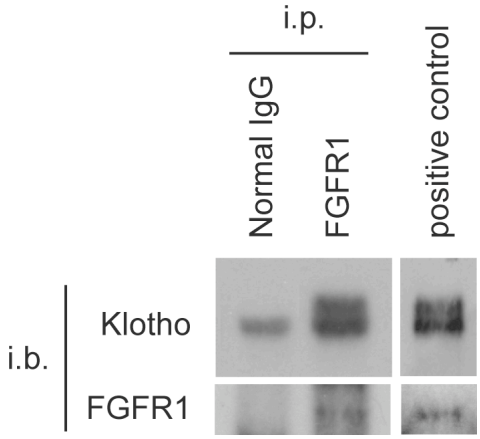
Exon#	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Supplemental Figure 1.
Splice variants of fibroblast growth factor receptors (FGFRs) used in this study. The FGFR1, FGFR2, and FGFR3 genes are composed of 19 exons[1]. Exon 3 encodes the first immunoglobulin (Ig)-like domain (Ig1) in the extracellular domain. Exons 8 and 9 alternatively encode the C-terminal half of the third Ig-like domain (Ig3). Exon 4 encodes the acid box and heparin-binding domain. Exclusion of exon 9 or exon 8 results in mRNA for "b" isoforms (b) or "c" isoforms (c), respectively. Inclusion of exon 3 and 4 encodes mRNA for "long" isoforms (L). Exclusion of exon 3 encodes mRNA for "middle" isoforms (M) in FGFR2 and for "short" isoforms (S) in FGFR1 and FGFR3. Exclusion of both exon 3 and 4 mRNA encodes "short" isoforms (S) in FGFR2. The FGFR4 gene is composed of 18 exons[2] and no splice variant has been described.

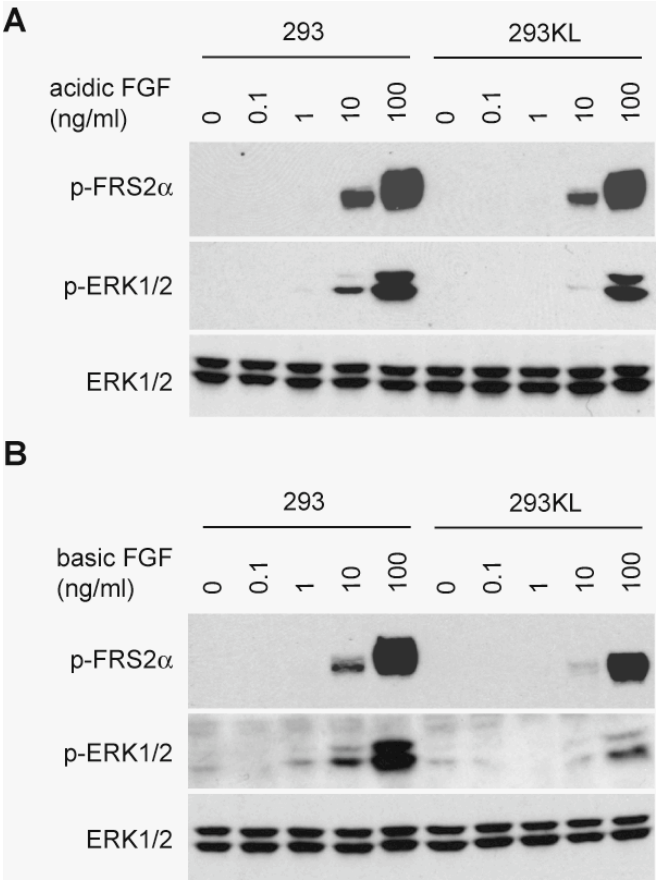
1 Givol, D., and Yayon, A. (1992) *Faseb J.* **6**, 3362-3369.
2 Kostrzewa, M., and Muller, U. (1998) *Mamm Genome.* **9**, 131-135.



Supplemental Figure 2.
Binding of the extracellular domain of Klotho to multiple FGFRs. Various FGFR isoforms were expressed in 293KLΔTM cells under the control of CMV promoter. Cell lysates were immunoprecipitated with Klotho using anti-Flag antibody and immunoblotted with FGFR using anti-V5 antibody (upper panel) or Klotho using KM2119 (center panel). To confirm even expression of exogenous FGFRs, the cell lysates were immunoblotted with FGFR using anti-V5 antibody (lower panel).



Supplemental Figure 3.
Co-precipitation of Klotho with FGFR1 from mouse kidney. Lysate of mouse kidney was immunoprecipitated (i.p.) with either anti-FGFR1 antibody or normal rabbit IgG as a negative control and then immunoblotted (i.b.) with either anti-Klotho antibody (KM2119) or anti-FGFR1 antibody. As a positive control for Klotho, lysate of mouse kidney was immunoprecipitated with another anti-Klotho antibody KL11-A and immunoblotted with KM2119 (upper right panel). As a positive control for FGFR1, lysate of 293 cells transfected with the mouse FGFR1c expression vector was immunoprecipitated with anti-V5 antibody and immunoblotted with anti-FGFR1 antibody (lower right panel). Klotho protein is detected as two bands by immunoblot analysis, representing different glycosylation (see Discussion). Although a non-specific band overlapped with the lower band of Klotho (upper left panel), the upper band of Klotho was co-precipitated specifically with FGFR1. This is consistent with the finding that the upper band of Klotho was enriched when co-precipitated with FGFR1c in the overexpression study using 293 cells (Fig. 1).



Supplemental Figure 4.
The activity of acidic FGF (**A**) and basic FGF (**B**) in activation of FGF signaling was compared between 293 and 293KL cells. Cell lysates were analyzed in the same way as Fig. 3A.